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No. 21 (1979)

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A. J. ELLIOTT.

LABORATORY INVESTIGATIONS INTO THE ABSORPTION OF DISSOLVED FREE AMINO ACIDS BY THE GILL OF THE MUSSEL *MYTILUS EDULIS* L.

Laboratory investigations into the absorption of dissolved free amino acids by the gill of the mussel *Mytilus edulis* L.

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ABSTRACT

Experiments were undertaken to investigate the uptake of dissolved free amino acids by the isolated gill of *Mytilus edulis* L., from concentrations approximating to those in sea water. Both neutral and basic amino acids were found to be absorbed against a concentration gradient and a proportion of each amino acid was incorporated in the tissue in ethanol-insoluble form. L-alanine was actively absorbed against a gradient by a process following Michaelis-Menten kinetics (Kt 0.33mM, Vmax 0.38μmoles/g dry wt/min) and was susceptible to metabolic inhibition. ^{14}C D-alanine was absorbed against a concentration gradient and the label incorporated in the tissue in ethanol-insoluble form. The fate of the absorbed amino acids is considered in relation to efflux from and metabolism in the mussel. The significance of amino acid uptake is discussed with reference to their availability in the environment and their contribution to the nutrition of bivalves.

INTRODUCTION

The common mussel *Mytilus edulis* L., though widely distributed in the northern hemisphere and plentiful on all coasts, is found in commercial size and quantity only in sheltered estuaries and inlets which are rich in organic nutrients. While organic particulate suspended matter in the water probably accounts for much of the growth of these mussels, it has recently been shown that the sea water in estuaries contains considerable quantities of dissolved organic nutrients including amino acids (Stephens 1963, 1972; Clark *et al.*, 1972). Uptake of dissolved nutrients has now been described in a large number of invertebrates and the literature has been reviewed by Jørgensen (1976).

The large epithelial surface areas of the gill and mantle in lamellibranchs would appear to be ideal for direct nutrient absorption. As a consequence of the normal filter-feeding activities of the animals these surfaces are exposed to a considerable volume of water. Anderson and Bedford (1973) demonstrated uptake of ^{14}C glycine from solution by the clam *Rangia cuneata* and also by its isolated gill tissue. The uptake of ^{3}H glycine by whole *Mytilus edulis* was reported by Péquignat (1973) and by means of autoradiographs he showed that the tritium was fixed in the mantle, foot and gill epithelia. He concluded that this absorption could furnish the mussel with a significant portion of its diet provided that these nutrients were continually renewed. More recently a number of investigations using *in vitro* preparations of gills of *Cerastoderma edule,* (Bamford and McCrea, 1975), *Mya arenaria* (Stewart and Bamford, 1975), *Mytilus californianus* (Wright *et al.*, 1975) and *Mytilus edulis* (Bamford and Campbell, 1976) have demonstrated uptake by an active process.

The purpose of this investigation was to determine whether the gill of *Mytilus edulis* can absorb dissolved free amino acids, whether this absorption is an active process and whether it is nutritionally significant.

MATERIALS AND METHODS

Mussels collected from Sutton, Co. Dublin were maintained in aerated sea water at 10°C and equilibrated for at least 24 hours before use. None was kept longer than two weeks. For each experiment the right gills of several mussels were excised and transferred to artificial sea water (Lyman and Fleming, 1940). Each gill was incubated at 10°C in a shaken 25ml Ehrlenmeyer flask containing 10 ml of a known concentration of ^{14}C uniformly labelled substrate (10μCi/l; Radiochemical Centre, Amersham, England) made up in artificial sea water. Controls containing no tissue were run concurrently. After incubation the tissue was removed, washed in non-radioactive medium of the same substrate concentration and drained for a few seconds on a zinc grid. Samples of incubation media and controls were stored at 7°C in vials containing thymol.

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The tissue was weighed, extracted overnight in 2 ml of 80% ethanol, and for a further 3-6 hours in a second aliquot. The two extracts were pooled and stored at 7°C. They were used to measure the ethanol-soluble or 'free' radioactivity. The extracted tissue was dried overnight at 105°C and weighed. It was then hydrolysed in 4 ml of 1N KOH for at least 48 hours at room temperature, being shaken occasionally. The digest thus obtained was used to measure the ethanol-insoluble or 'incorporated' radioactivity.

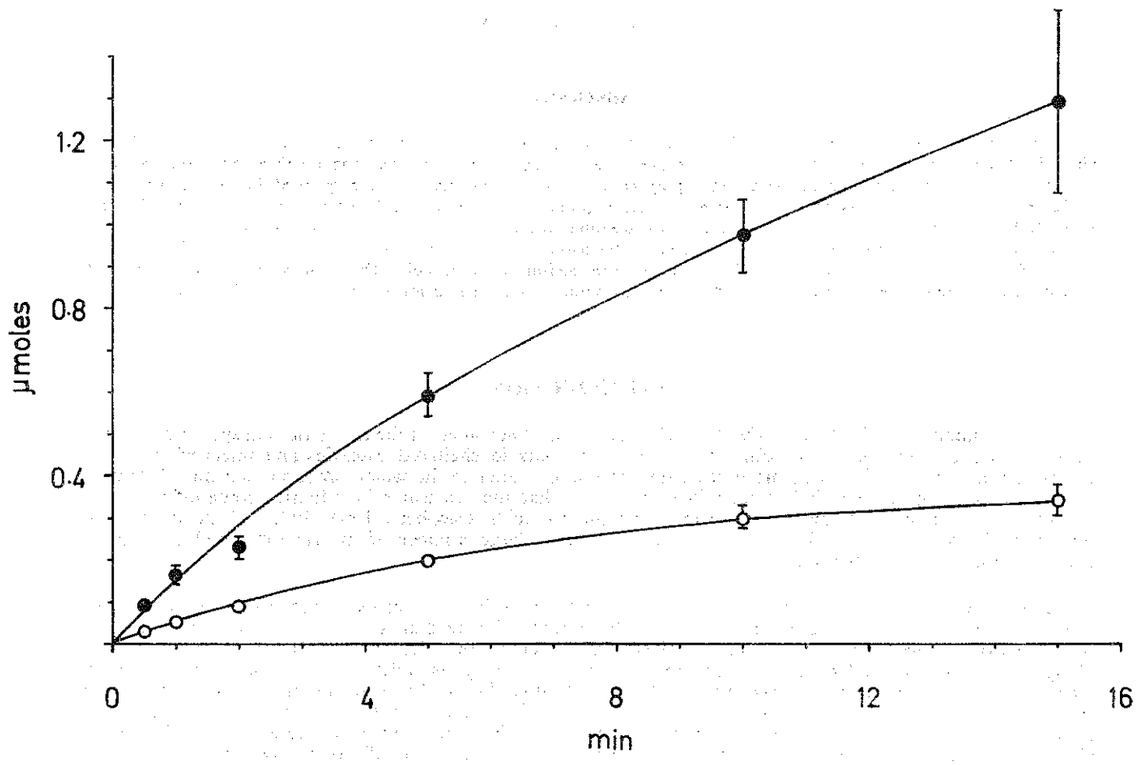

FIGURE 1. Uptake (in μmoles/g dry weight gill) of L-alan ine from 0.2mM solution as a function of time. Closed circles represent the ethanol soluble activity, open circles the incorporated activity. Each point is the mean of six replicates and the vertical bars represent the standard error.

The radioactivity of 1 ml aliquots of incubation media and ethanol extracts, and 0.5 ml aliquots of tissue hydrolysates neutralised with 0.5 ml of 1N HCl, were counted in 10 ml of liquid scintillation cocktail (1000 ml toluene; 500 ml Triton X—100; 4.0g P.P.O.; 0.1g P.O.P.O.P.) using a Packard Tri-Carb 2002 Liquid Scintillation Counter, for 10 minute periods. Controls containing scintillant alone were run to estimate background radioactivity. The efficiencies of counting the sea water media, ethanol extracts and digests were consistent and insignificantly different from one another in the range used. Consequently it was unnecessary to calculate D.P.M. (disintegrations per minute) and all data are based on C.P.M. (counts per minute). The amount of substrate absorbed by the tissue present in ethanol-soluble form, in ethanol-insoluble form, and the concentration ratio (concentration of free label in the tissue to that in the incubation medium) were calculated for each experiment.

For chromatographic analysis pooled samples of the ethanol extracts for each incubation time and a control sample of pure ^{14}C amino acid were spotted onto Whatman's No. 1 chromatograph paper and run for 7-10 hours at room temperature using N-butanol, pyridine, water (1 : 1 : 1) as a solvent. After development the chromatograms were cut into strips from the baseline to the solvent front and the strips cut into 17 or 18 equal sections which were counted for radioactivity. The activity in each section was expressed as a percentage of the total in the strip and compared to the control.

RESULTS

Absorption of neutral and charged amino acids

The absorption of a number of neutral and charged amino acids at 0.02 mM concentration was examined over 10 minute periods of incubation (Table 1). Significant uptake and incorporation of neutral L-amino acids glycine, leucine, alanine and proline had occurred in the gill. The final concentration ratios in all these cases are well in excess of unity. The basic amino acid L-lysine exhibited uptake with a mean concentration ratio of 4.5. The acidic amino acid L-glutamate did not however attain a significant concentration ratio (1.4) which is also reflected in the low level of uptake and incorporation.

Chromatographic analysis of the tissue extract after the 10 minute incubations indicated that, with the exceptions of proline and glutamate, most of the radioactivity remained in its original form. Possibly as little as 31% of the glutamate label remained unaltered by the end of the incubation.

Absorption of L-alanine

The uptake of L-alanine (0.019 mM) was investigated after 30 and 60 minute periods of incubation. The results confirm that the uptake of ethanol-soluble ^{14}C alanine is considerable over a one hour period, and the high concentration ratios suggest that active processes are involved (Table 2). As the uptake of 0.02mM L-alanine is linear with respect to time only over a two minute period (Fig. 1) an incubation time of one minute was chosen for subsequent kinetic experiments.

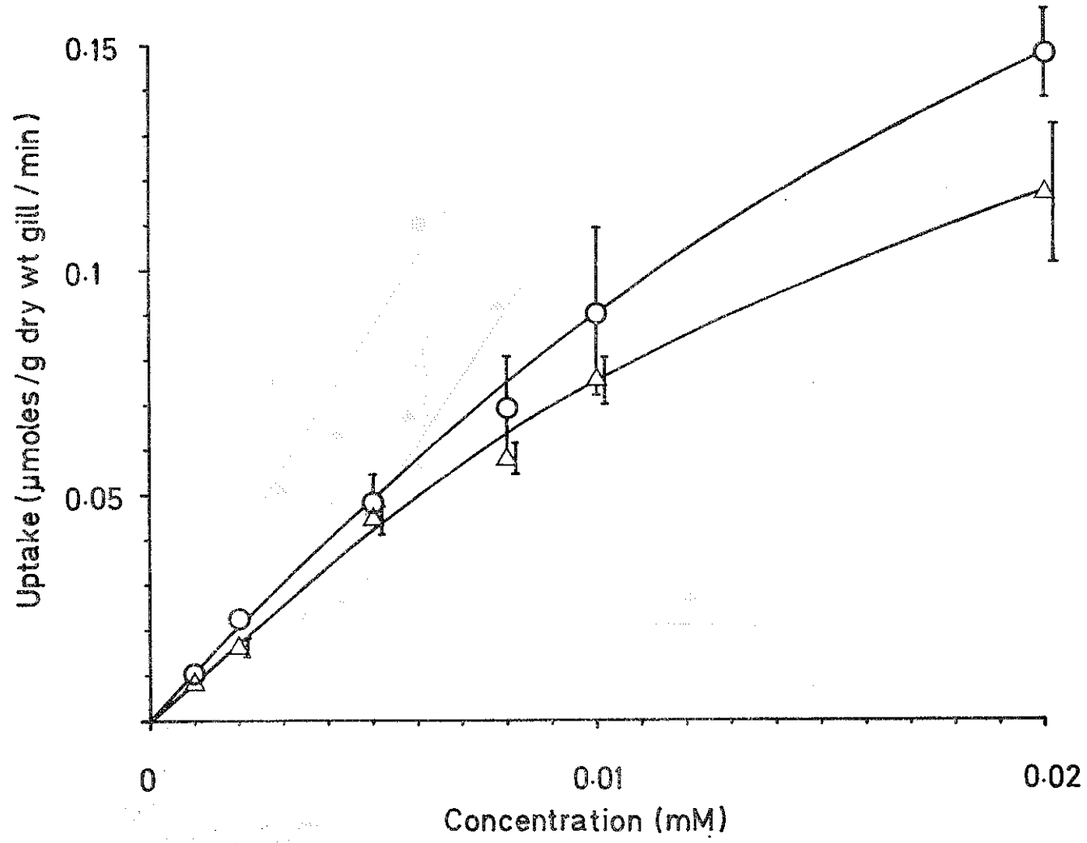

FIGURE 2. Absorption of free L-alanine as a function of concentration alone (circles) and in the presence (triangles) of 0.008mM glycine. All points represent the mean of six replicates and the vertical bars the standard error.

Uptake of L-alanine at concentrations ranging from 0.001 to 0.02 mM was determined in a series of 1 minute incubations (Fig. 2). The results were analysed in terms of Michaelis-Menten kinetics assuming diffusion to be negligible and using the Hofstee linear transformation (Dowd and Riggs, 1965). Values for Kt of 0.033 mM and for Vmax of 0.38 μmoles/g dry wt/min, were obtained (Fig. 3).

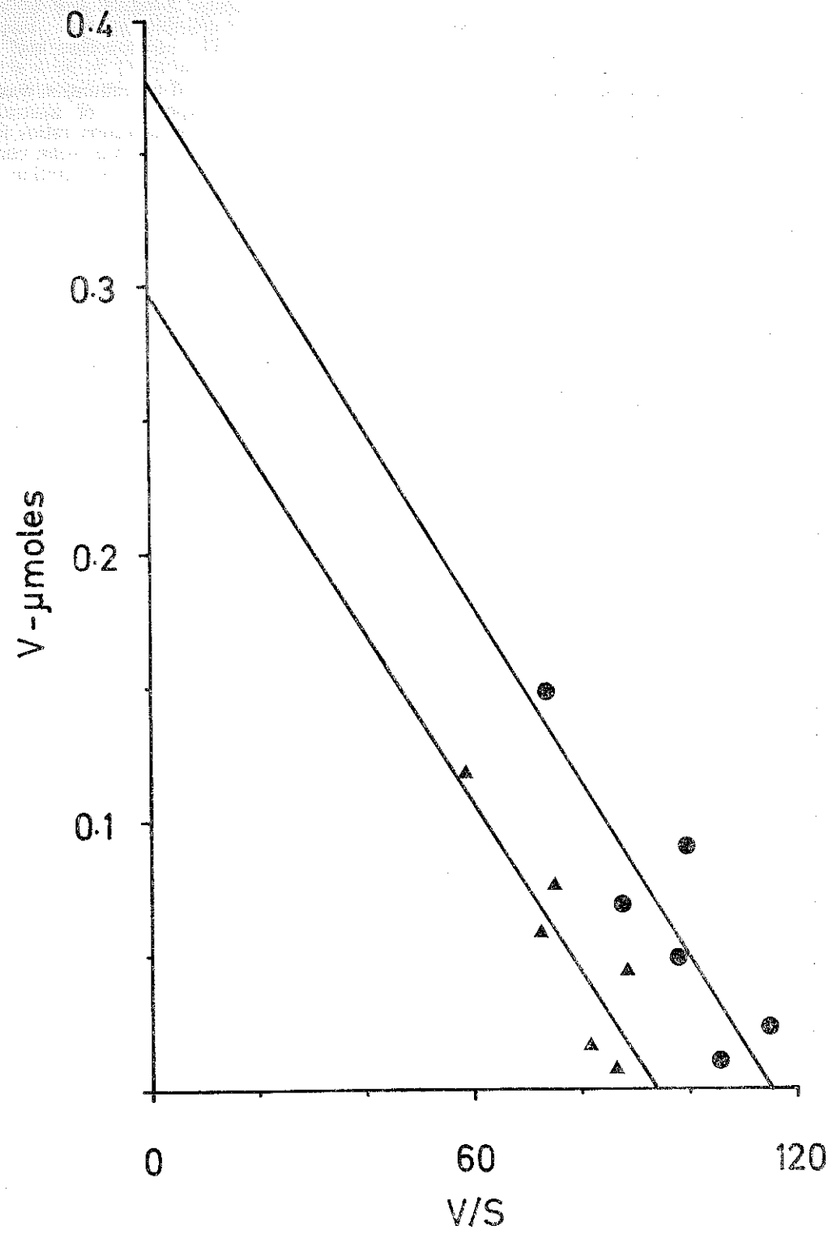

FIGURE 3. Kinetic analysis of L-alanine absorption alone (circles) and in the presence (triangles) of 0.008mM glycine. V = the velocity of alanine uptake in μmoles/g dry wt gill/min; S = substrate concentration in mM. Each point is the mean of six replicates. Regression lines calculated by the least squares method.

Effect of metabolic inhibitors on alanine absorption

The absence of uptake when energy yielding reactions are inhibited distinguishes active transport from facilitated diffusion, as energy from cell metabolism is not required in the latter. The effect of a number of

compounds, known to be metabolic inhibitors in vertebrate tissue, on the absorption of 0.02 mM L-alanine was investigated (Table 3). The inhibition of free alanine uptake varied from 21% to 89%. Uranyl nitrate was the most effective inhibitor and sodium iodacetate (NaIoA) the least. The effect of the inhibitors on incorporation was more variable, with maleic acid the most effective (67%) and NaCN the least (10%). Phlorizin actually increased incorporation when compared to the control.

Effect of other amino acids on alanine absorption

The effects of other neutral and basic acids (0.2mM) on the uptake of 0.02 mM L-alanine were examined in a series of one minute incubation experiments (Fig. 4). The neutral amino acids phenylalanine and methionine inhibited alanine uptake by about 40%. However serine and glycine were less effective inhibiting uptake by only 18% and 23% respectively. The basic amino acid L-lysine was the least effective depressing uptake by only 15%.

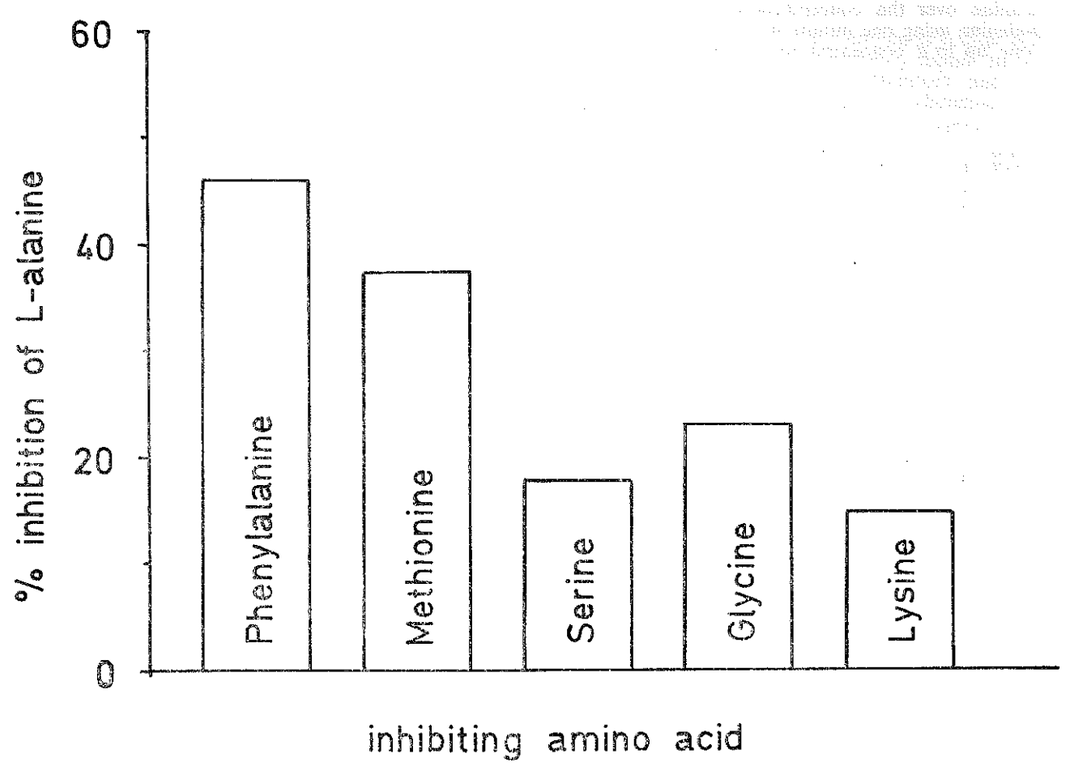

FIGURE 4. The effect of (0.2mM) neutral and basic amino acids on the uptake of free 0.02mM L-alanine. Each histogram represents the mean of six replicates. Incubation time 1 minute.

The inhibitory effect of glycine on alanine uptake was slight in view of its similar chemical configuration. In an attempt to elucidate the nature of this inhibition, the uptake of L-alanine over the concentration range 0.001-0.02 mM was measured in the presence of a fixed concentration of glycine (0.008 mM). Absorption was lower than was experienced with alanine alone (Fig. 2). When the two are compared using the Hofstee plot (Fig. 3), the lines are clearly parallel. Thus similar values for Kt are obtained but different values for Vmax i.e. inhibition is non-competitive (Dixon and Webb, 1964).

The neutral amino acid L-leucine is absorbed against a concentration gradient (Table 1) and might be expected to inhibit alanine uptake. To test this possibility uptake of 0.02 mM L-alanine was examined in the presence of increasing concentrations of L-leucine (Fig. 5). The increase from zero to 0.1 mM caused a rapid decrease in free alanine uptake. Thereafter the drop in uptake was slower till a maximum inhibition of 84% was achieved at 1.0 mM L-leucine.

Absorption of D-alanine

With the exception of D-methionine none of the D-amino acids exhibits transport against a concentration gradient in the mammalian small intestine (Wiseman, 1968), though Daniels *et al.* (1969) demonstrated inhibition of sarcosine transfer in rat small intestine by D-proline. Bamford and James (1972) and Stewart and Bamford (1975) among a number of other workers have reported uptake up D-alanine against a concentration gradient in invertebrate tissue.

Experiments were carried out to examine the absorption of D-alanine by the mussel gill (Table 4). Both ethanol-soluble and incorporated activity were detected. A concentration ratio well in excess of unity was achieved after 5 minutes incubation and at 15 minutes the mean concentration ratio was 7.5 $\pm$ 0.9. Chromatographic analysis of the ethanol extracts indicated that most of the radioactivity remained in its original form after absorption.

In an attempt to determine whether the transport locus for alanine showed stereospecificity the uptake of L-alanine over the concentration range 0.001 mM to 0.02 mM was observed in the presence of 0.008 mM D-alanine using one minute incubations (Table 5). Uptake was similar to that experienced with L-alanine alone up to a concentration of 0.01 mM of the L-isomer.

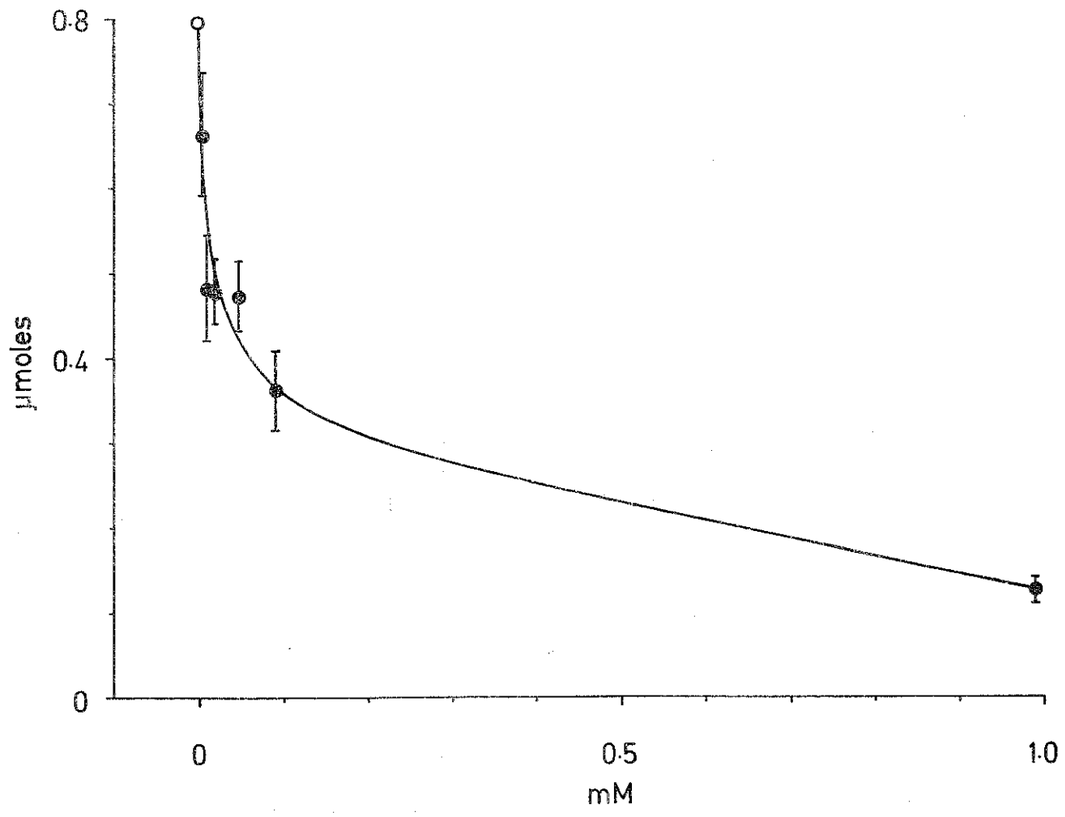

FIGURE 5. The effect of increasing concentrations of L-leucine on the uptake of free 0.02mM L-alanine Ordinate is the uptake of alanine in μmoles/g dry weight gill/10 min, abscissa the concentration of L-leucine. Each point represents the mean of seven replicates (open circle = 6 replicates) and the vertical bars the standard error.

DISCUSSION

Absorption of dissolved free amino acids

Uptake of both neutral and basic amino acids was found in the gill epithelium of *M.edulis* (Table 1). This supports evidence from elsewhere that the gills and other exposed surfaces (e.g. the mantle) of molluscs can absorb neutral and basic amino acids (Anderson and Bedford, 1973; Péquingnat, 1973; Bamford and McCrea, 1975; Stewart and Bamford, 1975; Wright *et al.*, 1975; Bamford and Campbell, 1976).

High concentration ratios were achieved for the neutral amino acids glycine, leucine and alanine in 10 minute incubations (Table 1). This suggests the presence of an active mechanism in the gill for the absorption of neutral amino acids at low substrate concentrations. Uptake of the heterocyclic amino acid L-proline and the basic amino acid L-lysine has been previously reported from the gut of the chiton, *Cryptochiton stelleri* by Greer and Lawrence (1967), and Bamford and McCrea (1975) found L-lysine uptake in the gill of the cockle, *Cerastoderma edule*.

L-glutamate (Table 1) does not appear to be actively absorbed by the gill. This finding is similar to that in a number of intestinal studies of acidic amino acid uptake. Greer and Lawrence (1967) found no active uptake of glutamate in *Cryptochiton stelleri* gut, and in *Echinus esculentus* stomach, Bamford and James (1972) found no evidence of active transport of L-aspartic acid. However, active uptake of glutamate occurs in whole polychaetes (Taylor, 1969; Chien *et al.*, 1972), and Bamford and Stewart (1973b) reported active absorption of L-aspartate by a saturable system in the intestine of *Arenicola marina*. Until recently the dicarboxylic amino acids were not considered to be transferred against a concentration gradient in mammalian intestine (Wiseman, 1968). However Schultz *et al.* (1970) have provided evidence of active acidic amino acid uptake across the brush border of rabbit ileum. Previously it had been considered that there was a rapid transamination of these amino acids during absorption which made the results difficult to interpret. This may be the case in the mussel gill (Table 1), for as Schultz *et al.* (1970) pointed out, failure to observe net transport of anionic amino acids against a concentration gradient cannot be interpreted as conclusive evidence against the presence of an active transport mechanism which could be obscured by extensive metabolism. Chromatographic analysis of the ethanol soluble fraction indicated that only about 30% of the radioactivity remained in the form of glutamate after 10 minutes of incubation.

Active uptake of alanine

L-alanine is absorbed by a saturable process conforming to Michaelis-Menten kinetics (Table 2, Figs. 2, 3). The Kt in mussel gill is of the same order as that observed for other naturally occurring neutral amino acids in other bivalve species (Table 6). However, lower values indicative of a higher affinity have been observed in the spines of the sea urchin *Paracentrotus lividus* and the pogonophore *Siboglinum ekmani*.

Alanine absorption was reduced in the presence of metabolic inhibitors, again suggesting that the process is an active one (Table 3). Similar effects have been observed in the gills of other bivalves (Stewart and Bamford, 1975; Bamford and McCrea, 1975) and on the uptake of phenylalanine in *Mytilus* gill by Bamford and Campbell (1976).

Chemically similar compounds usually have an inhibitory effect on one another in systems of active uptake. Both phenylalanine and methionine (Fig. 4) reduced L-alanine uptake by around 40% which is similar to their effect on alanine uptake in the gill of the cockle (Bamford and McCrea, 1975). However glycine appears to have a lesser effect on alanine uptake and the inhibition seems to be non-competitive in nature (Fig. 3). This would suggest that glycine utilises a separate pathway of active uptake in the gill. L-leucine has an inhibitory effect comparable to that of phenylalanine and methionine and alanine absorption was rapidly reduced on exposure to increasing concentrations of this substrate (Fig. 5). Bamford and McCrea (1975) and Stewart and Bamford (1975) have demonstrated significant inhibition of alanine uptake by L-leucine in the gills of *Cerastoderma* and *Mya* respectively. Thus the neutral amino acids, with the possible exception of glycine, may share a common pathway of absorption in the gill of the mussel.

The basic amino acid L-lysine had little inhibitory effect (15%) on the uptake of alanine (Fig. 4). This is somewhat at variance with the results observed in *Cerastoderma* (30%) and *Mya* (36%), (Bamford and McCrea, 1975; Stewart and Bamford, 1975). However Bamford and McCrea (1975) demonstrated that L-lysine was a non-competitive inhibitor of alanine uptake in *Cerastoderma* and was unlikely to share the same carrier site. It may be reasonable to suppose that L-lysine has a separate uptake pathway in *Mytilus* gill.

Absorption of D-alanine has previously been reported from the stomach of the sea urchin *Echinus* and the gut of the lugworm *Arenicola* (Bamford and James, 1972; James and Bamford, 1974; Bamford and Stewart, 1973a). More recently Stewart and Bamford (1975) have demonstrated active uptake of D-alanine by the gill of *Mya*. All these workers (with the exception of Bamford and Stewart, 1973a) have reported an apparent

stereospecificity in favour of the L-enantiomorph. Here the results (Tables 4, 5) are not as conclusive: 0.008 mM D-alanine had little effect on the L-alanine uptake though it appeared to stimulate absorption at higher substrate levels. Stewart and Bamford (1975) have suggested that some component of L-alanine uptake, in *Mya* gill, is not accessible to the D-form and conclude that more than one carrier site is involved. In *Arenicola* gut, Bamford and Stewart (1973a) suggested that separate carriers of each enantiomorph were involved. In *Mytilus* gill also there may be a number of carrier sites for alanine absorption at least one of which is inaccessible to the D-form.

Fate of the absorbed amino acids

On uptake from the medium it is assumed that the absorbed amino acids enter the intracellular free amino acid (FAA) pools (Stephens, 1968). Marine molluscs have high levels of FAA and their biochemistry has recently been reviewed by Campbell and Bishop (1970) and by Florkin and Bricteux-Gregoire (1972). Knowledge of the subsequent fate of the absorbed amino acids has been summarised by West *et al.* (1977).

One criticism of the present type of experiment has centred around the possibility of a net efflux of free amino acids from the tissue FAA pools (Johannes *et al.*, 1969; Johannes and Webb, 1970; Webb *et al.*, 1971). As much of this efflux would be unlabelled amino acid, due to the dilution factor, it would remain unmeasured. Stephens (1972) has given a detailed answer to the objections and Southward and Southward (1972) could find little substance in them.

To assess the possible loss of absorbed amino acids here, mussel gills were incubated in labelled 0.02 mM L-alanine for 30 or 60 minutes, removed, washed in 'cold' isomolar media, and reincubated in either unlabelled isomolar media or artificial sea water for a further 30 or 60 minutes. Controls were removed after the initial period and not reincubated. The results (Table 7) show that the label released from the tissue amounted to about 6% of the ethanol-soluble pool in the controls. This suggests that, despite the dilution factor, there is a net overall gain of amino acids from the medium and that loss of label in this manner is not significant.

Loss by the entry of labelled amino acids into respiratory pathways in the gill epithelial cells and emission of the radioactivity as $^{14}CO_2$ is another possibility. Both Stephens (1968) and Southward and Southward (1972) have considered it and de Burgh *et al.* (1977) found that 8% of total uptake of alanine in *Paracentrotus* spines was lost as $^{14}CO_2$. This process might account for the discrepancy between total absorbed activity in the experimental and the control tissues seen in Table 8.

Yet another loss from the FAA pools may come from incorporation into ethanol-insoluble organic compounds such as mucopolysaccharides and proteins. Experiments were conducted to assess the incorporation or adsorption of the label into gill mucus. Gills were incubated for 60 minutes in 0.02 mM L-alanine by which time sizeable quantities of mucus had accumulated. This was collected and the ethanol-soluble radioactivity determined in the usual manner. Controls were taken with the mucus included. The results (Table 9) suggest that alanine is accumulated in the mucus (with a concentration ratio of 5.4) but that this is insignificant when compared with the total alanine uptake of the gill. Péquignat (1973) has shown using autoradiographs that tritiated glycine is incorporated by *Mytilus* gill into mucus and released, and has described the subsequent uptake of this by the gut.

Apart from mucus a significant amount of the absorbed amino acid is incorporated in the tissue in ethanol-insoluble form. The present study reveals (Fig. 1, Tables 1, 3, 4, 7) that upwards of a third of the uptake is rapidly transferred into bound form. What the bound activity actually represents is uncertain but a proportion must reflect incorporation of the amino acids into polypeptides. Anderson and Bedford (1973) have attempted to analyse the incorporated activity in the clam *Rangia* after exposure to ^{14}C glycine. They report that (in sea water of salinity 10 and 15%) after three hours, 75% of the bound label was in protein, 20% in nucleic acids and 2% in both lipids and glycogen. De Burgh *et al.* (1977) have reported that virtually all the label in tissue digests of *Paracentrotus* spines, after incubation in ^{14}C L-alanine, was precipitated with trichloroacetic acid indicating that the ethanol-insoluble radioactivity had been incorporated into protein.

Role of absorbed amino acid in the energy budget

Absorbed free amino acids may play some role in the respiratory metabolism of the mussel. Southward and Southward (1972) have observed that total uptake may be underestimated by as much as 50% because only the amount of uptake present in the animal in soluble and insoluble form at the time of extraction is actually measured.

A number of workers have considered the uptake in terms of the energy budget (Stephens, 1962, 1963, 1968, 1972; Taylor, 1969; Southward and Southward, 1972; Anderson and Bedford, 1973). In discussing the nutritional significance of uptake the usual method has been to compare the resting or routine oxygen consumption of the animal with the amount of oxygen required to fully oxidise the substrate absorbed, assuming efficient deamination and aerobic oxidation.

The oxygen consumption of isolated mussel gill at 10°C has been found by La Touche (1974) to be 0.7 ml O_2/g dry wt/hr. It can be calculated that 1 ml of oxygen consumed is equivalent to the oxidation of approximately 1.3 mg of alanine. Thus the uptake of alanine (mg/g dry wt/hr) can be expressed as a percentage of the oxygen consumption (Table 10).

No data is available on the concentration of amino acids in the sea water over mussel beds though Clark *et al.* (1972) have reported total dissolved free amino acid concentrations from bottom water samples in the range 0.8-3.7μM. From Table 10 it can be seen that at a concentration of 10μM, alanine would meet 53% of the respiratory needs of the gill while at 1μM concentration it would meet 6%. Anderson and Bedford (1973) concluded that at a Kt of 0.046 mM the rate of glycine uptake by whole *Rangia* was equivalent to only 3.8% of its total oxygen requirement. Southward and Southward (1972) found that the pogonophore *Siboglinum ekmani* would be able to satisfy 50% of its respiratory needs from the maximum recorded concentration of amino acids in sea water. If amino acid concentration in the sediments is taken into account all the pogonophore's metabolic needs could be met. This is of significance because pogonophores have no internal digestive system and are therefore entirely dependent on epidermal absorption of nutrients either by active uptake or by pinocytosis and phagocytosis.

Caution should be exercised in interpreting this type of analysis. Uptake of dissolved free amino acids in mussel gill evidently occurs by an active process. This uptake may play a significant role in the synthetic and metabolic activities of the tissue, if not of the entire mussel. The results of this study do not imply that bivalves feed solely by absorption of dissolved organic nutrients, but they do indicate that dissolved amino acids can supply the animal with significant nourishment in addition to that derived from particulate food.

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Table 1. Absorption of L-amino acids at 0.02 mM initial concentration—10 min. incubations.

Values represent means of 6 replicates ± standard error.

Amino acid	Concentration ratio	Ethanol extract μmoles/g dry wt	KOH digest μmoles/g dry wt
Glycine	8.48 ± 2.42	0.91 ± 0.17	0.22 ± 0.03
L-leucine	7.40 ± 1.03	0.94 ± 0.12	0.16 ± 0.02
L-alanine*	8.64	0.92	0.21
L-proline	6.30 ± 0.44	0.66 ± 0.08	0.15 ± 0.02
L-lysine	4.46 ± 0.45	0.56 ± 0.04	0.19 ± 0.02
L-glutamate	1.45 ± 0.13	0.23 ± 0.03	0.09 ± 0.01

*3 replicates only.

Table 2. Absorption of 0.019 mM L-alanine as a function of time.

Values represent means ± S.E.

Time (min)	Concentration ratio	Ethanol extract μmoles/g dry wt	Replicates
30	62.10 ± 11.20	4.01 ± 0.57	4
60	120.09 ± 5.79	5.18 ± 0.62	6

Table 3. Effect of metabolic inhibitors on uptake of 0.02 mM L-alanine. 10 min. incubations—tissues pre-incubated for 60 min. in unlabelled isomolar media.

Results expressed as means of 6 replicates ± S.E.

Treatment	Inhibitor concn (mM)	Concentration ratio	Ethanol extract μmoles/g dry wt	Inhibition %	KOH digest μmoles/g dry wt	Inhibition %
Control	—	12.01 ± 2.03	0.82 ± 0.09	—	0.29 ± 0.03	—
KCN	2.0	3.44 ± 0.56	0.50 ± 0.06	39.0	0.22 ± 0.03	24.9
NaIoA	2.0	9.02 ± 1.96	0.64 ± 0.11	21.6	0.22 ± 0.05	24.1
Maleic acid	2.0	4.68 ± 1.75	0.39 ± 0.02	51.9	0.10 ± 0.01	66.8
Control	—	8.16 ± 0.30	0.62 ± 0.05	—	0.17 ± 0.01	—
NaCN	2.0	2.83 ± 0.39	0.40 ± 0.05	35.6	0.16 ± 0.01	10.0
Phlorizin	0.2	6.03 ± 0.95	0.45 ± 0.07	27.6	0.20 ± 0.03	+(14.5)
Uranyl nitrate	3.0	0.33 ± 0.02	0.07 ± 0.00	89.3	0.11 ± 0.00	34.5

Table 4. Absorption of 0.02 mM D-alanine as a function of time.

Values represent means of 6 replicates ± S.E.

Time (min)	Concentration ratio	Ethanol extract μmoles/g dry wt	KOH digest μmoles/g dry wt
0.5	0.403 ± 0.037	0.078 ± 0.007	0.017 ± 0.001
1	0.562 ± 0.036	0.107 ± 0.006	0.022 ± 0.002
2	1.241 ± 0.116	0.208 ± 0.022	0.030 ± 0.003
5	3.055 ± 0.454	0.549 ± 0.092	0.083 ± 0.010
10	5.482 ± 0.582	0.793 ± 0.090	0.140 ± 0.013
15	7.496 ± 0.884	0.986 ± 0.095	0.165 ± 0.014

Table 5. Absorption of L-alanine at varying concentrations in the presence and absence of 0.008 mM D-alanine. 1 min. incubations.

Values represent means of 6 replicates ± S.E.

Concentration (mM)	Concentration ratio + D-alanine	Ethanol extract + D-alanine μmoles/g dry wt	Ethanol extract control μmoles/g dry wt
0.001	1.191 ± 0.118	0.010 ± 0.001	0.010 ± 0.000
0.002	1.244 ± 0.091	0.021 ± 0.002	0.023 ± 0.002
0.005	1.167 ± 0.125	0.054 ± 0.006	0.049 ± 0.005
0.008	1.097 ± 0.126	0.078 ± 0.011	0.070 ± 0.010
0.01	1.043 ± 0.071	0.092 ± 0.009	0.091 ± 0.019
0.02	1.057 ± 0.194	0.185 ± 0.030	0.148 ± 0.010

Table 6. Kinetic data of amino acid uptake from various species.

Species	Tissue	Amino acid	Kt mM	Vmax μmoles/g dry wt/min
Siboglinum ekmani[1]	—	phenylalanine	0.003	*0.004
Paracentrotus lividus[2]	spine	alanine	0.008	0.027
Rangia cuneata[3]	—	glycine	0.046	0.045
Cerastoderma edule[4]	gill	alanine	0.024	0.042
Mya arenaria[5]	gill	alanine	0.095	0.527
Mya arenaria[6]	gill	histidine	0.08	0.3
Mytilus edulis[7]	gill	phenylalanine	0.012	0.272
Mytilus edulis[8]	gill	alanine	0.033	0.38

1. Southward and Southward (1970); 2. de Burgh *et al.* (1977); 3. Anderson and Bedford (1973); 4. Bamford and McCrea (1975); 5. Stewart and Bamford (1975); 6. Stewart and Bamford (1976); 7. Bamford and Campbell (1976); 8. This work.

* = g wet wt.

Table 7. Absorption and release of ^{14}C label from mussel gill incubated in 0.02 mM L-alanine for different times and reincubated for similar periods in unlabelled media.

Results expressed as means of 10 replicates ± S.E.

Treatment	Time (min)	Concentration ratio	Ethanol extract μmoles/g dry wt	KOH digest μmoles/g dry wt	Released μmoles/g dry wt	As % of control ethanol extract
control	30	28.27 ± 3.36	1.63 ± 0.16	0.51 ± 0.04	—	—
reincubated	30	26.83 ± 3.08	1.33 ± 0.16	0.62 ± 0.04	0.13 ± 0.01	7.7
control	60	126.38 ± 11.73	3.47 ± 0.29	0.92 ± 0.03	—	—
reincubated	60	90.95 ± 8.82	2.45 ± 0.18	0.99 ± 0.05	0.20 ± 0.01	5.7
control*	60	110.63 ± 11.54	3.76 ± 0.34	1.04 ± 0.11	—	—
reincubated*	60	137.26 ± 55.36	2.85 ± 0.40	1.23 ± 0.10	0.24 ± 0.02	6.5

*= Reincubated in sea water alone, 9 replicates only.

Table 8. Analysis of absorption and loss of radioactivity from mussel gill incubated in 0.02 mM L-alanine—^{14}C.

Reincubation media	Time (min)	Total absorbed label μmoles/g dry wt control	Total absorbed label μmoles/g dry wt reincubated	Label unaccounted for μmoles/g dry wt	% of control ethanol extract
L-alanine	30	2.14	2.07	0.07	4.6
L-alanine	60	4.39	3.64	0.75	21.5
sea water	60	4.80	4.33	0.47	12.6

Table 9. Accumulation of 0.02 mM L-alanine by gill mucus—60 min incubations. Values represent means ± S.E.

Treatment	Concentration ratio	Ethanol extract μmoles/g dry wt	KOH digest μmoles/g dry wt	Replicates
gill control	81.25	2.42	1.01	3
gill experimental	77.86 ± 5.29	2.35 ± 0.15	0.88 ± 0.05	9
mucus	*5.36 ± 0.72	**0.92 ± 0.08	—	9

* = $\frac{\text{CPM/ml mucus}}{\text{CPM/ml medium}}$

** = g dry wt mucus.

Table 10. Contribution of free L-alanine uptake to the energy budget of the mussel.

Substrate concentration μM	Alanine uptake mg/g dry wt/hr	Uptake as % of oxygen consumption
(Kt) 33	1.02	111.6
10	0.48	52.9
1	0.05	5.9

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